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Morphological and molecular changes following neoadjuvant endocrine therapy of ER positive breast cancer: implications for clinical practice

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Abstract

Background: Neoadjuvant endocrine therapy (NAET) is used in the management of estrogen receptor positive (ER+) breast cancer. The optimal method for histological assessment of response and the effect of NAET on the tumour morphology, grade and molecular profile remain unclear.

Material and methods: A single large institution cohort of 132 patients who received NAET over a 13-year period was identified. Comprehensive clinical, histopathological and follow up data were collected. A detailed histological review of a subset with residual post-treatment carcinoma was undertaken.

Results: Two carcinomas (both of lobular type) achieved complete pathological response. Central scarring was seen in 49.3% of tumours post-treatment. Significant changes in tumour type (41.6%), grade (downgrading in a third of tumours), PR expression (22.3%) with a switch to PR negative status in 17.6% of cases were observed. The latter was associated with absence of tumour infiltrating lymphocytes (p=0.005). 10% of cases showed a change in HER2 expression (p=0.002). Median patient survival was 60 months and downgrading of tumours was associated with better overall survival (p=0.05).

Conclusions: We propose a histological method for assessment of residual carcinoma following NAET and recommend repeat ER/PR/HER2 testing to inform management and prognosis.

Keywords

Neoadjuvant endocrine therapy (NAET), aromatase inhibitors, breast carcinoma, pathological response.

List of abbreviations

AIs: Aromatase Inhibitors
BC: Breast Cancer
ER: Estrogen Receptor
ET: Endocrine Therapy
FISH: Fluorescence In-Situ Hybridization
NAET: Neoadjuvant Endocrine Therapy
NST: No special type
OS: Overall Survival
pCR: Pathological Complete Response

PEPI: Pre-operative Endocrine Prognostic Index

PR: Progesterone Receptor

TILs: Tumour Infiltrating Lymphocytes

Introduction

Endocrine therapy is increasingly being used upfront for the treatment of breast cancer either as primary therapy for patients unsuitable for chemotherapy/surgery [1], [2] or as a neoadjuvant treatment option for hormone receptor positive breast cancer. Neoadjuvant endocrine therapy (NAET) can reduce tumour size to allow breast conservation and also influence the management decision on chemotherapy [3]. Patients with hormone receptor positive breast cancer showed overall clinical response rates ranging from 36-51% for tamoxifen and 38-70% for aromatase inhibitors (AIs) [4-11]. A UK multicenter audit of neoadjuvant therapies showed considerable variation in the uptake rate and practice of NAET with patchy information on the pathological response rate [12]. During the COVID pandemic, patients with ER positive HER2 negative early breast cancer received neoadjuvant endocrine therapy to postpone surgical treatment that was prioritized for the more urgent and aggressive tumours such as triple negative and HER2 positive cancers [13, 14]. Therefore, it has become essential for pathologists and the multidisciplinary teams to be familiar with the handling, reporting and management of patients undergoing NAET.

Currently, the pre-operative endocrine prognostic index (PEPI) is the only available index that relates the response to therapy and risk of relapse but is not currently in use in routine practice. It is based on the assessment of tumour size, nodal status, Ki67 level and ER Allred score. Patients with low pathological stage and a favorable biomarker profile (PEPI score 0) showed a lower rate of relapse indicating that adjuvant chemotherapy can be omitted compared with those with high pathological stage disease at surgery and a poor biomarker profile [3]. There is sparsity of data on the histological changes that follow NAET regarding tumour profile and hormonal receptor expression. Unlike neoadjuvant chemotherapy, there are no guidelines to assess pathological response after NAET and neoadjuvant chemotherapy reporting systems are not validated for use in the endocrine setting. Therefore, there is inconsistency of assessment and histological reporting of post-neoadjuvant endocrine tumours [15]. In this study, we aim to investigate the NAET effect on tumour type, grade, and molecular profile through analyzing a well characterized cohort of tumour samples in a single large UK tertiary referral center and provide guidance on the pathological assessment of those lesions to inform adjuvant management and prognosis.

Patients and methods

Female patients who underwent NAET for invasive breast carcinoma at Queen Elizabeth Hospital Birmingham, UK were identified from the clinical databases.

Study group

Included in this study were patients with primary ER positive operable breast carcinoma who received NAET followed by breast surgery in the period between November 2007 and December 2019. The treatment was standardised as per the national and local protocols and the average duration of treatment was 6 months. Patients who did not undergo surgery (primary endocrine therapy) were excluded. Surgical specimens were sampled thoroughly similar to the neoadjuvant chemotherapy specimen sampling. Where possible, a marker clip was inserted before therapy to indicate the site of the tumour. Where no tumour could be identified macroscopically, specimen x-raying was performed to identify a marker clip, radiological calcification and/or residual tumour.

Data collection

Comprehensive clinical data including patients' age, ethnicity, type of NAET treatment, type of surgery and overall survival (OS) data were collected. The following pathological data, where available, were identified from the pathology reports on both pre-treatment core biopsy samples and residual tumours: tumour type, tumour grade including individual scores for tubule formation, nuclear pleomorphism and mitoses, estrogen (ER) and progesterone receptor (PR) status, HER2 status and pathological response. Immunohistochemistry score of 3+ or 2+ fluorescence in situ hybridization (FISH) amplified were defined as HER2 positive as per the UK guidelines [16]. The response was classified into pathological complete response (pCR) (no residual invasive carcinoma in breast and axillary nodes), minimal residual disease (≤ 10% residual invasive carcinoma), pathological partial response (residual invasive carcinoma comprising >10% with histological evidence of tumour response), and no response (no evidence of tumour response). A detailed histological review of tumour morphology, grade, tumour cellularity (assessment of percentage of average cancer cellularity across the largest cross section of the residual tumor bed), scarring pattern (central versus diffuse fibrosis) with central scarring defined as central acellular fibrous area surrounded by residual tumour cells [15], margin status (pushing vs infiltrating), architecture of the tumour (nodular vs diffuse) was undertaken on 75 of post-operative tumour sections by two pathologists (NMB, AMS), including a specialist breast pathologist. Tumour infiltrating lymphocytes (TILs) were also assessed in the residual post treatment carcinoma following the International Immuno-Oncology Biomarker Working Group on Breast Cancer guidelines. Stromal TILs (TILs that were not in direct contact with the tumour nests or cells) were evaluated. Immune infiltrates outside of the tumour borders, e.g.in adjacent normal tissue or DCIS were not be included in the assessment [17].

Immunohistochemical staining

Immunohistochemical staining was performed for the characteristic areas of tumours from microscopically selected samples (regions), based on examining the standard (hematoxylin and eosin) H&E staining. De waxing of the slides in PT link DAKO automated immunohistochemistry system is processed for hormonal receptors and in Roche Ventana Ultra machine for HER2 staining. The slides were processed in target retrieval solution for 70 minutes at 97 C (Cell Conditioning solution 1(CC1) for 64 minutes at 95 C in HER2 staining). CC1 next blocking step was performed using peroxidase inhibiter preceded and followed by washing. The slides were incubated in the primary antibody (ER; clone EP1, DAKO, RTU, PR; clone PgR 1294, DAKO, RTU and HER2; clone 4D5. After wash, slides were incubated in FLEX/HRP for 30 minutes blocking step and FLEX DAB for 10 minutes, each with a wash before and after (in HER2 staining, HRP multi timer was used instead for 8 minutes blocking step (Roche, antibody diluent with casein) and DAB was added for 8 minutes with wash before and after that followed by copper sulphate solution for 4 minutes). Lastly embedding the slides into Hematoxylin was done for 15 minutes.

Statistical analysis

Statistical analysis was done using the IBM SPSS package V.24. Analysis for pre- and post-treatment categorical variables including tumour type, grade, ER/PR Allred score and HER2 expression was done using Chi-square test. Receptor status was also dichotomised into negative and positive using a cut-off value of Allred score >2 for ER/ PR and Her2 immunohistochemical score of 3+ (or 2+ fluorescence in situ hybridization (FISH) positive) to define positivity. The non-parametric Kruskal Wallis test was used to assess the relation between cellularity as a continuous variable and response to therapy. The Kaplan-Meier method was used for survival analysis. Overall survival was defined as the duration in months between the date of diagnosis and the date of last follow-up or death. A p-value of ≤ 0.05 was considered significant.

Results

A total of 132 patients fulfilled the inclusion criteria. The neoadjuvant regimen was predominantly aromatase inhibitors (AIs, 96.2%) with the rest of patients receiving Tamoxifen. The pre-treatment clinicopathological characteristics of all patients are summarised in table 1. The majority (96.2 %) of the patients were aged over 50 with a median age of 73 years (range: 40 - 93 years) and were mostly Caucasian (84.1%). On pre-treatment biopsy, the tumours were predominantly of no special type carcinoma (NST, 63.6%) and grade 2 differentiation (74.1%). All tumours were ER

positive; of which 88.6% were PR positive and 6.8% HER2 positive. The few HER2 positive patients were administered neoadjuvant endocrine therapy and not chemotherapy or anti- HER2 therapy either due to age/comorbidities or patient choice. Pathological complete response (pCR) was achieved in two cases (2.6%) while 18.2%, 75.3% and 3.9% of patients showed minimal residual disease, pathological partial response, and no pathological response, respectively. The two cases that achieved pCR were of patients of Caucasian ethnicity; aged 74 and 58 years. Both were diagnosed with invasive classical lobular carcinoma of grade 2, hormone receptor strongly positive (Allred scores for ER were 8/8 for both, PR scores were 6/8 and 7/8, respectively) with negative HER2 status.

Of note, tumours that received AIs were statistically significantly more likely to show histological evidence of tumour response compared with the Tamoxifen group (97.2% vs 75%, p=0.03). Tumour stage 2 (ypT2) was seen in 64.8% of the patients. Breast conserving surgery was achieved in 67.4% of patients. Sentinel lymph node biopsy was performed in 68.8% with nodal metastasis observed in 49.6% of patients.

Histological tumour type

The two invasive carcinomas that showed pCR were of the lobular type. There was a change in the histological type following NAET in 55 out of 132 cases (41.6%) and this was statistically highly significant (p=0.001) with an increase in tubular carcinoma by 3% and a decrease in the mixed subtypes by 1.5%. Details of the histological types before and after NAET are shown in table 2.

Tumour grade

Table 3 summarises tumour grades pre- and post-NAET. Downgrading (decrease in the overall grade by at least one grade) was observed in 30.4 % of cases, and up-grading (increase in overall grade by at least one grade) in 15.6% (p=0.01). The downgrading was predominantly due to a decrease in the mitotic count ($p<0.001$) and an increase in tubule formation (p= 0.05) and was significantly associated with better survival. Grade 2 and 3 tumours comprised 85.9% of cases on pre-treatment core biopsies. This proportion decreased to 69.5% following treatment. The proportion of grade 1 carcinoma increased from 14% to 30.4% post-NAET.

Histological features of response to NAET

Of the 75 available post-treatment surgical excisions, 77.3% showed infiltrative tumour margins. Central scarring was seen in 49.3% of cases (Fig. 1a). Tumour cellularity ranged from 1 to 90%

with an average of 40.6%. Lymphocytic infiltration was noted in 25.3% of cases. A nodular architecture of the tumour comprising multiple adjacent foci was noticed in 32% of cases (Table 4). Reduced tumour cellularity was significantly related to achieving response to therapy ($p<0.001$) (Fig. 1b, 1c).

ER expression

Following treatment, one out of 130 available tumour pairs (0.7%) changed profile from ER positive to negative. Five cases (Allred scores 6 and 7) showed an increase in Allred score by at least one score. Ten cases (Allred score 8) showed reduced ER expression by at least one score.

PR expression

The PR status showed a highly significant change between pre- and post-treatment tumour samples in 22.3% of cases ($p <0.001$). Twenty-three cases (17.6%) changed from PR positive to negative status (Fig.1d, 1e) and five changed from negative to positive (3.8%). Further variation in the Allred score without affecting the final PR status was noted in sixty-nine cases (52.2%) (table 5). Tumours that retained the same PR status following therapy correlated with better response to therapy ($p=0.018$). Decrease of PR Allred score was significantly associated with absence of lymphocytic infiltration ($p=0.005$).

HER2 expression

One hundred and fourteen HER2 negative (87.7%) and another three HER2 positive (2.3%) tumours retained the same profile following NAET treatment. A discordant HER2 status following treatment was noted in 13 out of 130 cases (10%). Five cases changed profile from HER2 overexpression to HER2 negative (3.8%). Eight cases changed from negative to positive status following treatment (6.1%). This change was statistically highly significant ($p = 0.002$).

Lymph node response

Pre-treatment lymph nodes were assessed by imaging and cytology/biopsy sampling. Following NAET, 49.6% of tumours showed histologically confirmed nodal metastasis. The nodal metastasis showed similar histological features of regression to the breast carcinoma particularly the associated fibrosis. No significant association between nodal status and the patient outcome was found in this cohort.

Patient survival

Overall survival ranged from eight to 137 months with a median of 60 months (Inter quartile range (IQR):36-84, 95% CI, 57-67). Downgrading of tumours following NAET was associated with

better overall patient survival (p=0.05). Patients with no change in their PR status following NAET had longer mean survival time than those whose tumours that lost PR expression following treatment (107.3 months and 91.7 months) respectively. However, this difference did not reach statistical significance. No significant association was found between other histological parameters and patient overall survival.

Discussion

Little is known about the effect of neoadjuvant endocrine therapy (NAET) on breast tumour characteristics. In this study, we report significant changes in the histological tumour type, grade, cellularity, and receptor status following NAET. We show that tumours tended to acquire more specialized phenotypes with a change of 21/84 of NST carcinomas (25%) to other special tumour types including tubular, lobular, mucinous and/or mixed types. Downgrading of invasive carcinoma was associated with better overall survival.

The variation in the histological type, grade, hormone receptor and HER2 status is intriguing. While this may reflect a genuine change in the tumour phenotype as a result of neoadjuvant endocrine therapy, the effects of sampling and/or tumour heterogeneity should also be considered. For example, the change of 8 lobular carcinomas and 3 mucinous carcinomas into no special type carcinoma is likely to reflect a pre- treatment mixed phenotype and/or tumour heterogeneity. It is plausible that the limited core biopsy sampled a different histological type/profile to the full excision. It is also possible that various tumour profiles responded differently to NAET with the least responsible/resistant phenotype remaining as residual carcinoma. We therefore recommend repeat testing of the residual carcinoma for ER/PR/HER2 as a switch from negative to positive result would provide treatment options for patients. The final molecular profile may be a better indicator of prognosis compared with the pre-treatment sample. In the current study, tumours that retained their PR profile were associated with better survival. A change in PR status from positive to negative may be an early indication of endocrine resistance.

NAET has an anti-proliferative effect on breast tumour cells, which is associated with reduced expression of the proliferation marker Ki67 in patients showing response to therapy irrespective of NAET regimen [18-20]. A previous study reported downgrading of the breast carcinomas following either AIs or tamoxifen. This was associated with fewer mitoses with AI therapy while tamoxifen induced more tubule formation [18]. Here, we report a significant reduction of the mitotic activity and more prominent tubule formation resulting in downgrading of one third of the tumours

following AIs. In addition, complete pathological response of two invasive lobular carcinomas was achieved. In the UK, Ki67 is not performed routinely on breast carcinomas with and without prior therapy as per the NHS Breast Screening Programme (NHSBSP) guidelines [21]

In the current study, a minor effect of NAET on ER expression was noted with only one case switching to an ER negative status. The effect of NAET on ER expression had not been well documented in the literature and the data so far has been conflicting with reports suggesting a reduction [19] [20] or no significant effect following neoadjuvant AIs [22].

Data from the IMPACT trial showed a reduction in PR levels by 41% and 82% at 2 and 12 weeks of anastrazole therapy respectively while tamoxifen resulted in its increased expression at 2 weeks followed by a return to pre-treatment levels at 12 weeks [22]. A reduction in PR expression levels following NAET has also been documented in previous studies [18, 23]. It has long been thought that PR positivity is a pre-requisite for a favourable response to endocrine therapy [24]. Progesterone receptor status significantly improved outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases [24] and is considered an indication of an intact ER signaling pathway [25, 26]. Subsequently, PR was shown to be downregulated by growth factors indicating it is a potential surrogate for breast cancer tumour activity [25]. The IMPACT trial showed more marked reduction of ki67 levels in the PR positive tumours [22]. Our reported decrease in PR levels in 69.8% of cases following treatment may be an indication of suppression of the ER signaling pathway and less likelihood for further tumour response to endocrine therapy compared with tumours that retain PR expression. Although there was no correlation between this change and overall patient survival, we observed that patients with no change in their tumour PR status had longer mean survival time than those that lost PR expression following NAET. This might be a reflection of the poor prognostic effect of loss of PR expression following NAET.

The change in HER2 expression following neoadjuvant endocrine therapy is a novel finding. A cross talk between the ER and HER2 pathways has been described [27] and a change in ER/PR/HER2 expression following neoadjuvant chemotherapy has been well documented [28, 29]. The precise mechanisms by which residual tumours change profile remain unknown. A recent concept of transcriptional plasticity/adaptation to allow tumour cells to escape the treatment effects has been proposed [30]. HER2 signaling members were shown to reduce ER expression

both at the mRNA and protein levels [31]. ER was also shown to stimulate down-regulation of the HER1 and HER2 expression [32, 33]. Thus, a combination of the AIs and trastuzumab correlated with a longer progression-free survival compared with AIs alone in the HER2 positive tumours [34]. In the neoadjuvant setting, potent anti-HER2 treatment with trastuzumab and lapatinib combined with letrozole in patients with locally advanced HER2 positive/ ER positive breast carcinoma resulted in pCR rate of 21% [35]. A long follow up for those patients that showed a change in tumour profile is warranted to confirm the clinical significance of this finding.

The rate of pCR reported in this study is remarkably low. pCR in the neoadjuvant endocrine setting has uniformly been reported to be less than 10% and this was confirmed in a recent meta-analysis [36]. Morphological changes of tumour regression were evident following NAET and included decreased tumour cellularity and increased fibrosis [15, 18]. In this current study, central fibrous scarring was a frequent finding, identified in almost half of the cases. Thomas et al., were first to document the appearance of central scarring in 58.5% of their cohort treated with hormonal therapy as compared with systemic chemotherapy (4%). In their study, central scarring was associated with clinical reduction of tumour volume [15]. We did not find a correlation between central scarring and the pathological response to therapy or patient survival.

Although lymphocytic infiltration was not a common feature following NAET, we observed that the absence of lymphocytic infiltrate was significantly related to decreased PR expression in post-treatment samples. This highlights a potential link between the tumour immune response and PR suggesting a prognostic role for TILs in the neoadjuvant endocrine setting. Infiltrating immune cells in pre-treatment cores were shown to be associated with poor response to neoadjuvant AIs. [37].

Downgrading of tumours correlated with longer patient overall survival. However, no significant correlation was found between other studied clinical or histological parameters and survival in our cohort. As per a comprehensive meta-analysis of neoadjuvant studies, correlation with survival as an end point for NAET studies may be challenging. Possible reasons include the recommended use of adjuvant endocrine therapy with variable adherence, potential use of adjuvant chemotherapy, and the long indolent course of the ER positive tumours and their low early recurrence rate [36].

There are some similarities and differences in the histological response following NACT and NAET. While some histological feature, such as fibrosis, decreased cellularity and lymphocytic infiltrate are common to both neoadjuvant chemotherapy and neoadjuvant endocrine response, we confirmed that central scarring was characteristic of NAET response and is seen in approximately half of the cases while this feature is uncommon following NACT. This observation was first reported by Thomas et al 2008 [15] and confirmed in this study. The changes in tumour morphology, grade, ER/PR/HER2 status have previously been reported following NACT in several studies. However, the proportion of carcinomas showing ER conversion (from positive to negative) following NACT (5.7%[38], 12.4%[39], 5.2%[28]) is much higher than that noted after NAET in the current study (one case, 0.77%) .

In conclusion, we report significant changes in tumour morphology, PR and HER2 expression following neoadjuvant endocrine therapy. Downgrading of invasive carcinomas post-treatment is associated with better survival. Central fibrous scarring is a common finding and lymphocytic infiltration seems to play a role in association with the decreased PR expression following therapy. The data highlight the importance of thorough histological assessment of the post-treatment surgical specimens. We recommend detailed pathological examination of the residual post-NAET tumours including tumour grade, presence/absence of central scarring, lymphocytic infiltrate, and repeat hormone receptor and HER2 testing (table 6). The changes in hormone receptor and HER2 status may be prognostic and predictive of response to therapies that otherwise would not be offered to patients based on the pre-treatment tumour profile. Standardized reporting of those increasingly encountered complex specimens is required to allow comparison of results among institutions globally and to help collect high quality histological and outcome data to inform future patient management.

Conflict of interest statement

None

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Table 1

Clinicopathological features of the study cohort (pre-treatment)

Criterion	**NO (%)**
Age	
>50	127 (96.2%)
≤ 50	5 (3.8%)
Ethnicity	
Caucasian	111 (84.1%)
Asian	11 (8.3%)
Black	3 (2.3%)
Other	7 (5.3%)
Invasive tumour type	
No special type (NST)	84 (63.6%)
Lobular carcinoma	22 (16.7%)
Other (mucinous, tubular) carcinoma	9 (6.8%)
Mixed ductal and lobular carcinoma	17 (12.9%)
Tumour grade*	
I	18 (13.7%)
II	97 (74.1%)
III	16 (12.2%)
Progesterone receptor (PR)	
Positive	117 (88.6%)

Negative	15 (11.4%)
HER2	
Positive	9 (6.8%)
Negative	123 (93.2%)
Tumour stage**	
T1	30 (24.5%)
T2	79 (64.8%)
T3	13 (10.7%)
Lymph node stage***	
N0	63 (50.4%)
N1	39 (31.2%)
N2	12 (9.6%)
N3	11 (8.8%)
Type of treatment	
Aromatase inhibitors	127 (96.2%)
Tamoxifen	5 (3.8%)
Type of surgery	
Breast conserving surgery	89 (67.4%)
Mastectomy	43 (32.6%)

*Grading was not applicable in one core due to tiny amount of tumour.

**Tumour size was assessed radiologically before treatment (data for 10 cases were not available)

*** Data of 7 cases were not available.

Table 2

Details of tumour type pre and post-neoadjuvant endocrine therapy

Pre-NAET tumour type	Post-NAET tumour type						
	NST carcinoma	Lobular carcinoma	Mucinous carcinoma	Tubular carcinoma	Mixed carcinoma	No residual	Total
NST carcinoma	63	4	3	6	8	0	84
Lobular carcinoma	8	10	0	0	2	2	22
Mucinous carcinoma	3	1	1	0	1	0	6
Tubular carcinoma	1	1	0	0	1	0	3
Mixed carcinoma	11	2	0	1	3	0	17
Total	86	18	4	7	15	2	132

NAET: Neoadjuvant endocrine therapy

NST: No special type carcinoma

Table 3

The distribution of tumour grade in pre-treatment core biopsy and post-treatment residual invasive carcinoma

Pre-NAET grade		Post-NAET grade			Total
		I	II	III	
	I	11	6	1	18
	II	27	54	13	94
	III	1	11	4	16
Total		39	71	18	128*

NAET: Neoadjuvant endocrine therapy

*Two specimens had no residual disease and grading was not applicable in further two due to the tiny amount of tumour in one core and one residual disease.

Table 4

Details of the histological findings of 75 residual carcinoma post-neoadjuvant endocrine treatment

Parameter	**NO (%)**
Scarring pattern	
Central	37(49.3%)
Diffuse	38(50.7%)
Tumour margin	
Infiltrative	58 (77.3%)
Well circumscribed	17(22.7%)
Architecture of the tumour	
Nodular	24(32%)
Diffuse	51(68%)
Lymphocytic infiltration	
Positive	19(25.3%)
Negative	56(74.7%)
Pathological response*	
Pathological complete response (pCR)	2(2.6%)
Minimal residual disease	14(18.2%)
Pathological partial response	58(75.3%)
No response	3(3.9%)

Table 5

PR Allred score pre- and post-neoadjuvant endocrine treatment

PR pre-NAET		PR post-NAET									Total
		0	1	3	4	5	6	7	8	NA	
	0	10	0	0	1	1	1	0	1	0	14
	2	0	0	1	0	0	0	0	0	0	1
	3	0	0	2	0	0	0	0	1	0	3
	4	1	0	0	2	0	2	0	0	0	5
	5	3	0	0	1	6	1	0	1	0	12
	6	1	0	1	2	1	1	1	1	1	9
	7	5	0	1	2	1	1	1	0	1	12
	8	13	1	7	5	11	8	4	27	0	76
Total		33	1	12	13	20	14	6	31	2	132

PR: Progesterone receptor, NAET: Neoadjuvant endocrine therapy.

NA: Not applicable, two cases achieved pathological complete response.

Table 6: Recommended microscopic features to include in the histological reporting of post-neoadjuvant endocrine therapy tumours.

Histological features
Tumour Type
Tumour grade
Tumour margin: pushing/infiltrative
Central scarring: present/absent
Tumour cellularity: (%)
Tumour infiltrating lymphocytes [17]
Repeat receptor testing
Estrogen receptor (ER)
Progesterone receptor (PR)
HER2
Pathological response
Pathological complete response (pCR)
Minimal residual disease: (≤ 10% residual invasive carcinoma with evidence of tumour response)
Partial response (PR): (>10% residual invasive carcinoma with evidence of tumour response)
No histological evidence of response

Figure legends

Figure 1: Histological features of response to neoadjuvant endocrine therapy

1a. Post-treatment surgical excision showing central fibrous scarring with peripheral viable tumour cells.

1b. High tumour cellularity pre-treatment.

1c. Low tumour cellularity post-treatment, same tumour.

1d. PR positive invasive no special type carcinoma (pre-treatment).

1e. The tumour switched to a PR negative status following neoadjuvant endocrine therapy.

References

1. Mathew J, Asgeirsson KS, Jackson LR, Cheung KL, Robertson JFR. Neoadjuvant endocrine treatment in primary breast cancer – Review of literature. The Breast. 2009;18(6):339-44.
2. Barroso-Sousa R, Silva DDAFR, Alessi JVM, Mano MS. Neoadjuvant endocrine therapy in breast cancer: current role and future perspectives. Ecancermedicalscience. 2016;10:609-.
3. Ellis MJ, Tao Y, Luo J, A'Hern R, Evans DB, Bhatnagar AS, et al. Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. J Natl Cancer Inst. 2008;100(19):1380-8.
4. Eiermann W, Paepke S, Appfelstaedt J, Llombart-Cussac A, Eremin J, Vinholes J, et al. Preoperative treatment of postmenopausal breast cancer patients with letrozole: A randomized double-blind multicenter study. Ann Oncol. 2001;12(11):1527-32.
5. Smith IE, Dowsett M, Ebbs SR, Dixon JM, Skene A, Blohmer JU, et al. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(22):5108-16.
6. Fontein DB, Charehbili A, Nortier JW, Meershoek-Klein Kranenbarg E, Kroep JR, Putter H, et al. Efficacy of six month neoadjuvant endocrine therapy in postmenopausal, hormone receptor-positive breast cancer patients--a phase II trial. Eur J Cancer. 2014;50(13):2190-200.
7. Kaufmann M, von Minckwitz G, Mamounas EP, Cameron D, Carey LA, Cristofanilli M, et al. Recommendations from an international consensus conference on the current status and future of neoadjuvant systemic therapy in primary breast cancer. Ann Surg Oncol. 2012;19(5):1508-16.
8. Semiglazov VF, Semiglazov VV, Dashyan GA, Ziltsova EK, Ivanov VG, Bozhok AA, et al. Phase 2 randomized trial of primary endocrine therapy versus chemotherapy in postmenopausal patients with estrogen receptor-positive breast cancer. Cancer. 2007;110(2):244-54.
9. Cataliotti L, Buzdar AU, Noguchi S, Bines J, Takatsuka Y, Petrakova K, et al. Comparison of anastrozole versus tamoxifen as preoperative therapy in postmenopausal women with hormone receptor-positive breast cancer: the Pre-Operative "Arimidex" Compared to Tamoxifen (PROACT) trial. Cancer. 2006;106(10):2095-103.
10. Masuda N, Sagara Y, Kinoshita T, Iwata H, Nakamura S, Yanagita Y, et al. Neoadjuvant anastrozole versus tamoxifen in patients receiving goserelin for premenopausal breast cancer (STAGE): a double-blind, randomised phase 3 trial. Lancet Oncol. 2012;13(4):345-52.

11. Santa-Maria CA, Camp M, Cimino-Mathews A, Harvey S, Wright J, Stearns V. Neoadjuvant Therapy for Early-Stage Breast Cancer: Current Practice, Controversies, and Future Directions. Oncology (Williston Park). 2015;29(11):828-38.

12. Ian Whitehead, Gareth Irwin, Finian Bannon, Charlotte Coles, Ellen Copson, Ramsey Cutress, et al. The NeST (Neoadjuvant Systemic Therapy in Breast Cancer Study): National Practice Questionnaire of United Kingdom Multidisciplinary Decision Making. Research square. 2020.

13. https://associationofbreastsurgery.org.uk/media/296462/abs-ukbcg-statement-may-20-final.pdf. Accessed September 2020.

14. https://www.breastsurgeons.org/docs/statements/ASBrS-Resource-Guide-on-Endocrine-Therapy-in-the-COVID-19-Pandemic.pdf. Accessed September 2020.

15. Thomas JS, Julian HS, Green RV, Cameron DA, Dixon MJ. Histopathology of breast carcinoma following neoadjuvant systemic therapy: a common association between letrozole therapy and central scarring. Histopathology. 2007;51(2):219-26.

16. Rakha EA, Pinder SE, Bartlett JMS, Ibrahim M, Starczynski J, Carder PJ, et al. Updated UK Recommendations for HER2 assessment in breast cancer. 2015;68(2):93-9.

17. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Annals of oncology : official journal of the European Society for Medical Oncology. 2015;26(2):259-71.

18. Miller WR, Dixon JM, Macfarlane L, Cameron D, Anderson TJ. Pathological features of breast cancer response following neoadjuvant treatment with either letrozole or tamoxifen. Eur J Cancer. 2003;39(4):462-8.

19. Harper-Wynne CL, Sacks NP, Shenton K, MacNeill FA, Sauven P, Laidlaw IJ, et al. Comparison of the systemic and intratumoral effects of tamoxifen and the aromatase inhibitor vorozole in postmenopausal patients with primary breast cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2002;20(4):1026-35.

20. Makris A, Powles TJ, Allred DC, Ashley S, Ormerod MG, Titley JC, et al. Changes in hormone receptors and proliferation markers in tamoxifen treated breast cancer patients and the relationship with response. Breast cancer research and treatment. 1998;48(1):11-20.

21. Ellis I, Al-Sam S, Anderson N, Carder P, Deb R, Girling A, et al. Pathology reporting of breast disease in surgical excision specimens incorporating the dataset for histological reporting of breast cancer, June 2016. 2019.

22. Dowsett M, Ebbs SR, Dixon JM, Skene A, Griffith C, Boeddinghaus I, et al. Biomarker changes during neoadjuvant anastrozole, tamoxifen, or the combination: influence of hormonal status and HER-2 in breast cancer--a study from the IMPACT trialists. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(11):2477-92.

23. Maeda S. Changes of estrogen receptor, progesterone receptor and HER2 expression in breast cancer with/without neoadjuvant chemotherapy or endocrine therapy. Journal of Clinical Oncology. 2019;37(15_suppl):e14699-e.

24. Bardou VJ, Arpino G, Elledge RM, Osborne CK, Clark GM. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2003;21(10):1973-9.

25. Cui X, Zhang P, Deng W, Oesterreich S, Lu Y, Mills GB, et al. Insulin-like growth factor-I inhibits progesterone receptor expression in breast cancer cells via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathway: progesterone receptor as a potential indicator of growth factor activity in breast cancer. Molecular endocrinology (Baltimore, Md). 2003;17(4):575-88.

26. Jensen EV, DesSombre ER. Steroid hormone binding and hormone receptors, in Holland JF, Frei E III, Bast RC Jr, et al (eds). Cancer Medicine, 4th edition. 181996. p. pp 1049-60

27. Giuliano M, Trivedi MV, Schiff R. Bidirectional Crosstalk between the Estrogen Receptor and Human Epidermal Growth Factor Receptor 2 Signaling Pathways in Breast Cancer: Molecular Basis and Clinical Implications. Breast Care (Basel). 2013;8(4):256-62.

28. Gahlaut R, Bennett A, Fatayer H, Dall BJ, Sharma N, Velikova G, et al. Effect of neoadjuvant chemotherapy on breast cancer phenotype, ER/PR and HER2 expression - Implications for the practising oncologist. European journal of cancer (Oxford, England : 1990). 2016;60:40-8.

29. Rossi L, Verrico M, Tomao S, Ricci F, Fontana A, Spinelli GP, et al. Expression of ER, PgR, HER-2, and Ki-67 in core biopsies and in definitive histological specimens in patients with locally advanced breast cancer treated with neoadjuvant chemotherapy. Cancer Chemother Pharmacol. 2019.

30. da Silva-Diz V, Lorenzo-Sanz L, Bernat-Peguera A, Lopez-Cerda M, Munoz P. Cancer cell plasticity: Impact on tumor progression and therapy response. Semin Cancer Biol. 2018;53:48-58.

31. Guo S, Sonenshein GE. Forkhead box transcription factor FOXO3a regulates estrogen receptor alpha expression and is repressed by the Her-2/neu/phosphatidylinositol 3-kinase/Akt signaling pathway. Mol Cell Biol. 2004;24(19):8681-90.

32. Lee AV, Cui X, Oesterreich S. Cross-talk among estrogen receptor, epidermal growth factor, and insulin-like growth factor signaling in breast cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2001;7(12 Suppl):4429s-35s; discussion 11s-12s.

33. Newman SP, Bates NP, Vernimmen D, Parker MG, Hurst HC. Cofactor competition between the ligand-bound oestrogen receptor and an intron 1 enhancer leads to oestrogen repression of ERBB2 expression in breast cancer. Oncogene. 2000;19(4):490-7.

34. Kaufman B, Mackey JR, Clemens MR, Bapsy PP, Vaid A, Wardley A, et al. Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: results from the randomized phase III TAnDEM study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009;27(33):5529-37.

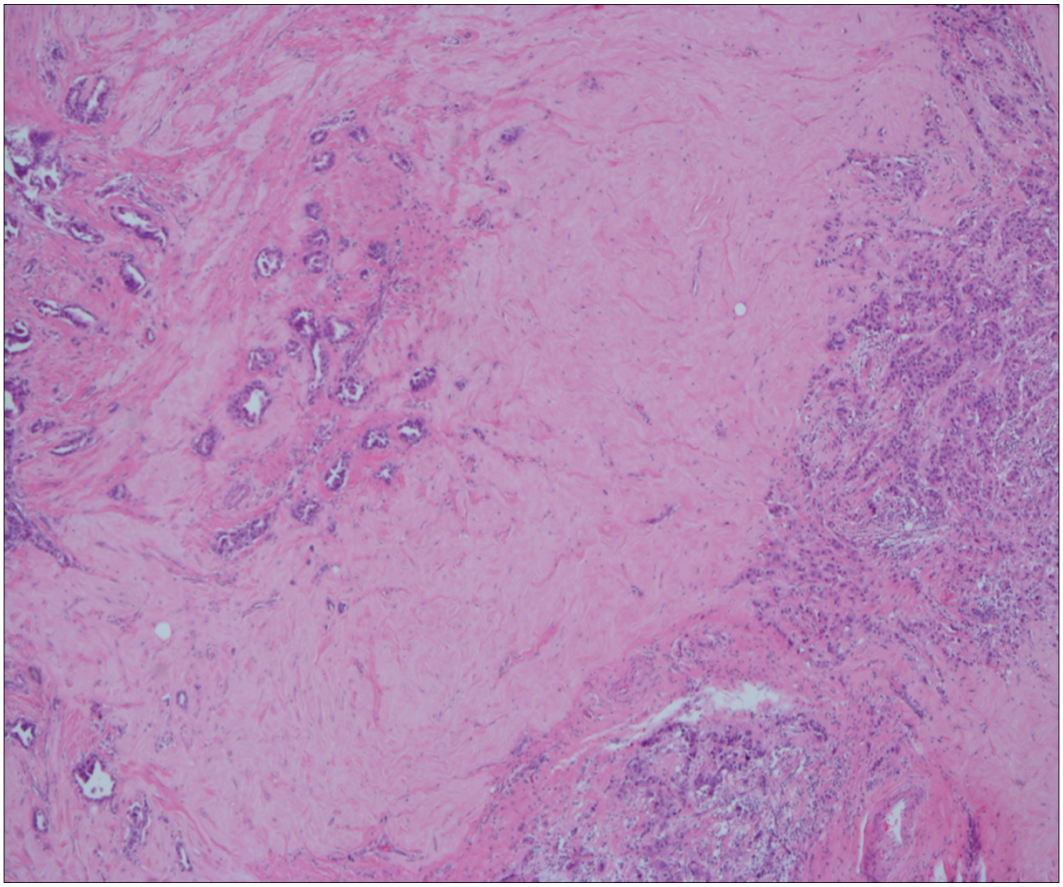
35. Rimawi MF, Mayer IA, Forero A, Nanda R, Goetz MP, Rodriguez AA, et al. Multicenter phase II study of neoadjuvant lapatinib and trastuzumab with hormonal therapy and without chemotherapy in patients with human epidermal growth factor receptor 2-overexpressing breast cancer: TBCRC 006. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31(14):1726-31.

36. Spring LM, Gupta A, Reynolds KL, Gadd MA, Ellisen LW, Isakoff SJ, et al. Neoadjuvant Endocrine Therapy for Estrogen Receptor-Positive Breast Cancer: A Systematic Review and Meta-analysis. JAMA Oncol. 2016;2(11):1477-86.

37. Dunbier AK, Ghazoui Z, Anderson H, Salter J, Nerurkar A, Osin P, et al. Molecular profiling of aromatase inhibitor-treated postmenopausal breast tumors identifies immune-related correlates of resistance. Clinical cancer research : an official journal of the American Association for Cancer Research. 2013;19(10):2775-86.

38. Dede DS, Gumuskaya B, Guler G, Onat D, Altundag K, Ozisik Y. Evaluation of changes in biologic markers ER, PR, HER 2 and Ki-67 index in breast cancer with administration of neoadjuvant dose dense doxorubicin, cyclophosphamide followed by paclitaxel chemotherapy. Journal of BUON : official journal of the Balkan Union of Oncology. 2013;18(2):366-71.

39. Yang YF, Liao YY, Li LQ, Xie SR, Xie YF, Peng NF. Changes in ER, PR and HER2 receptors status after neoadjuvant chemotherapy in breast cancer. Pathology, research and practice. 2013;209(12):797-802.



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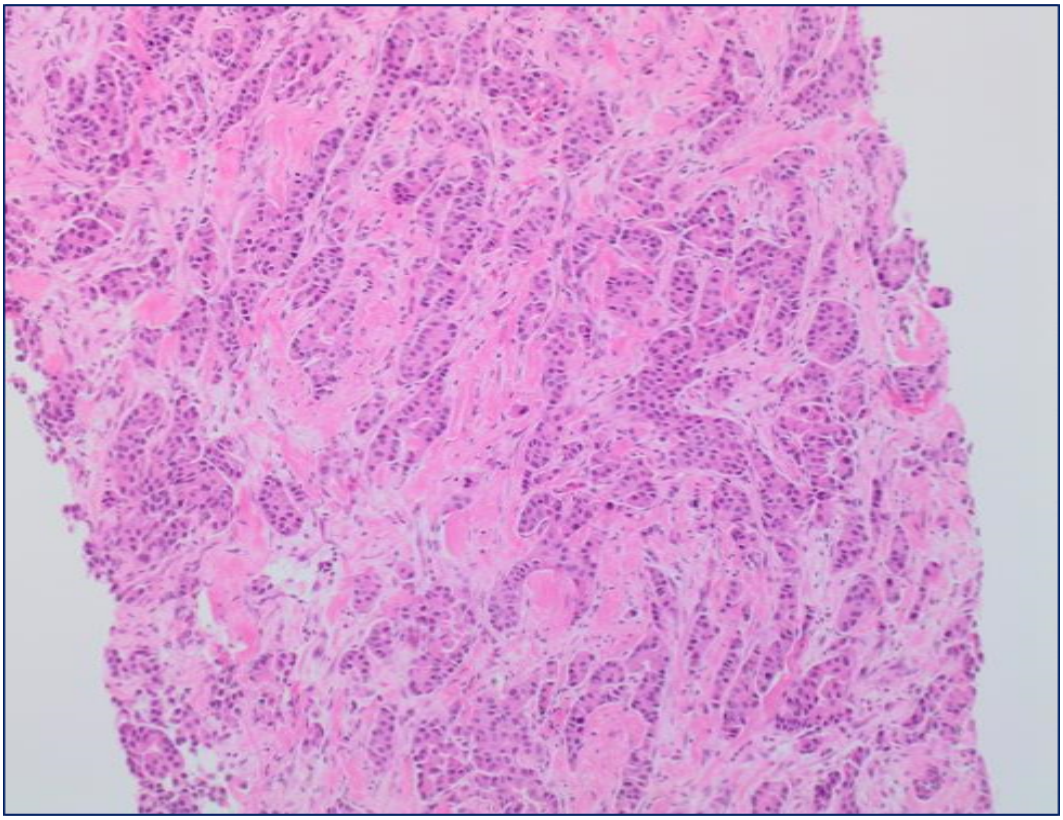

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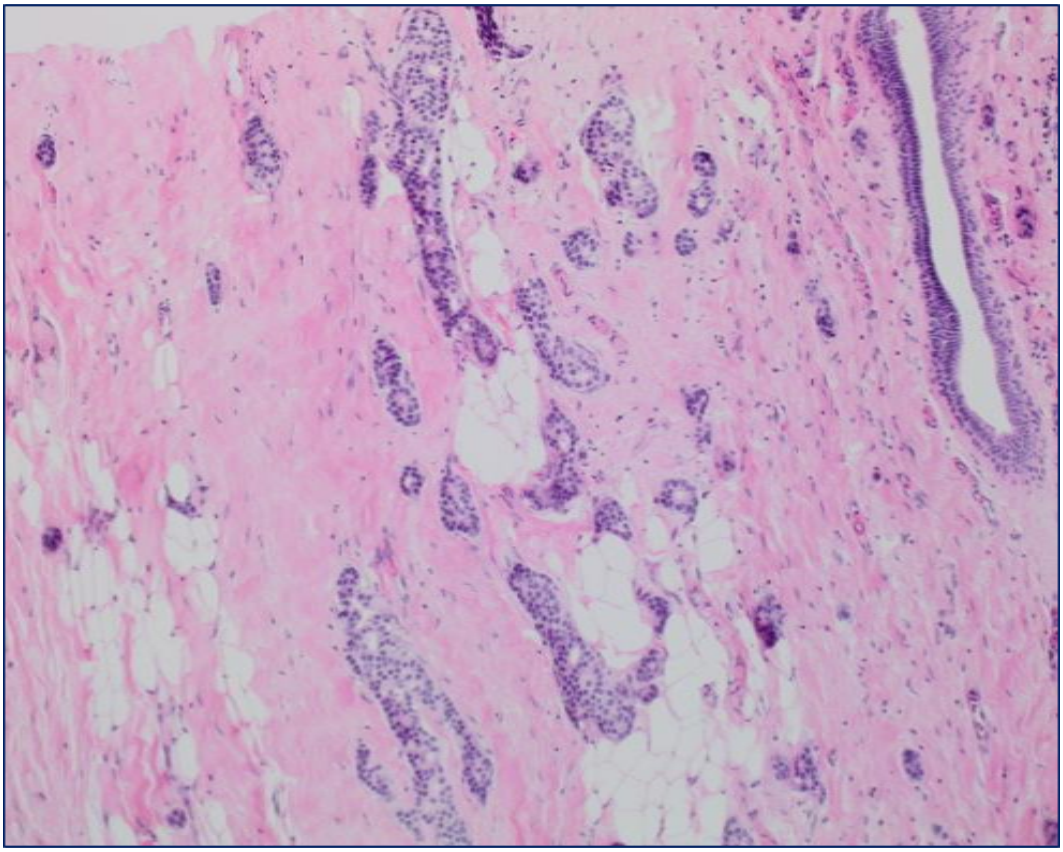

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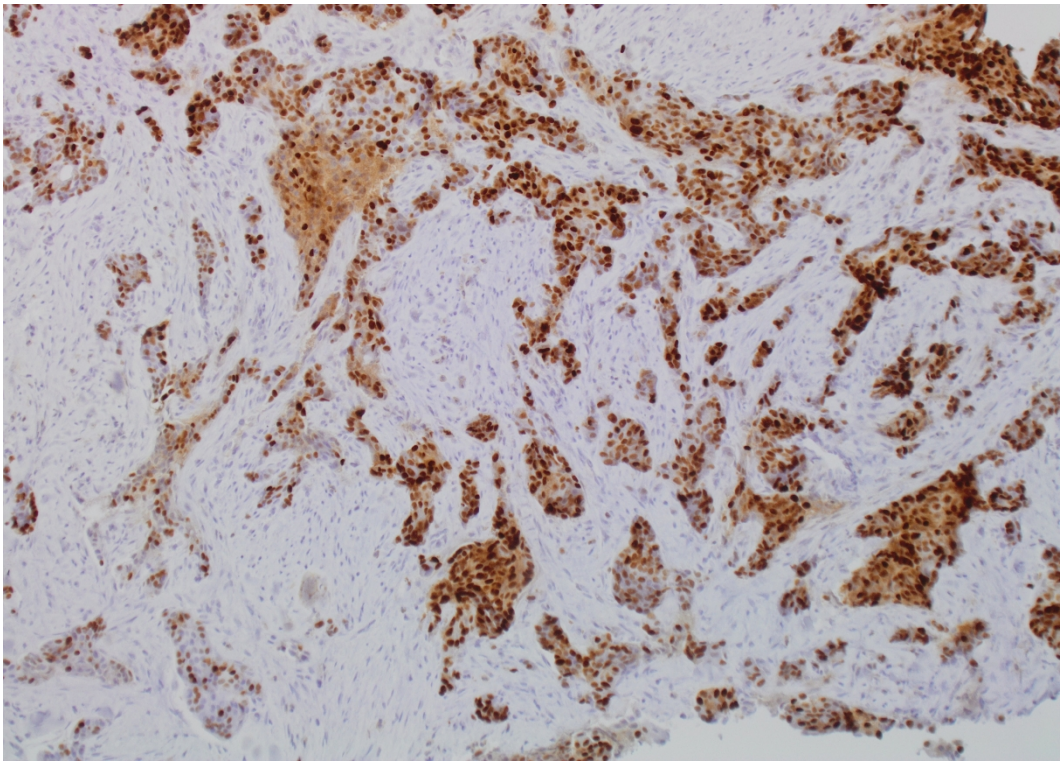

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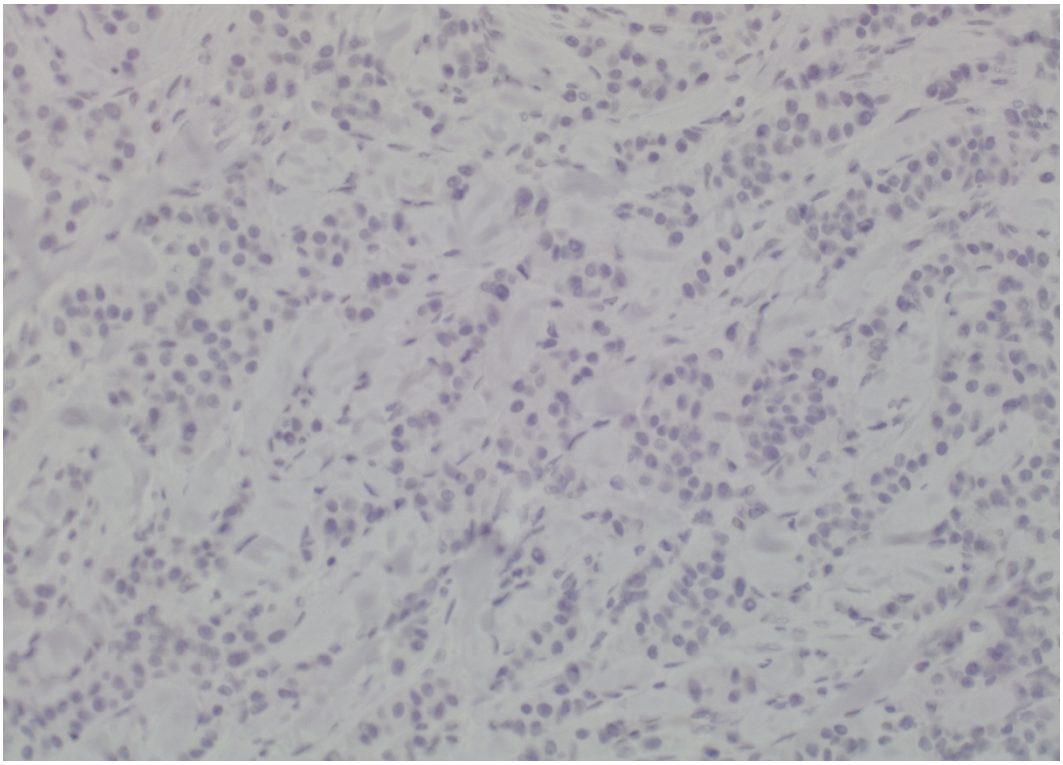

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